Gene Expression Analysis of a Down's Syndrome Study Using Partek® Genomics Suite™ 6.6

This tutorial will illustrate how to:

- Import Affymetrix® CEL files and check quality
- Add attributes describing the sample groups
- Perform exploratory analysis using the PCA scatter plot
- Find differentially expressed genes using ANOVA
- Generate a list of genes of interest
- Add annotations to the gene list

Note: the workflow described below is enabled in Partek® Genomics Suite™ version 6.6. Please contact the Partek Licensing Team at licensing@partek.com to request this version or update the software release via *Help > Check for Updates* from the main command line. The screenshots shown below may vary across platforms and across different versions of Partek® Genomics Suite™.

Description of the Data Set

Down syndrome is caused by an extra copy of all or part of chromosome 21; it is the most common non-lethal trisomy in humans. The study used in this tutorial revealed a significant up-regulation of chromosome 21 genes at the gene expression level in individuals with Down syndrome; this dysregulation was largely specific to chromosome 21 only and not to any other chromosomes. This experiment was performed using the Affymetrix® GeneChip™ Human U133A arrays. It includes 25 samples taken from 10 human subjects and 4 different tissues.

The raw data for this study is available as experiment number GSE1397 in the Gene Expression Omnibus: http://www.ncbi.nlm.nih.gov/geo/.

Data and associated files for this tutorial can be downloaded by going to **Help > On-line Tutorials** from the Partek® Genomics Suite™ main menu. The data can also be downloaded directly from: http://www.partek.com/Tutorials/microarray/Gene_Expression/Down_Syndrome/Down_Syndrome-GE.zip.

Importing Affymetrix® CEL Files

Download the data from the Partek® site to your local disk.

- For this tutorial, unzip the files to C:\Partek Training Data\Down_Syndrome_GE or to a directory of your choosing. Be sure to create a directory or folder to hold the contents of the zip file.
- Start Partek® Genomics Suite™ and select **Gene Expression** from the *Workflows* panel on the right side of the tool bar in the Partek® Genomics Suite™ main window (Figure 1)

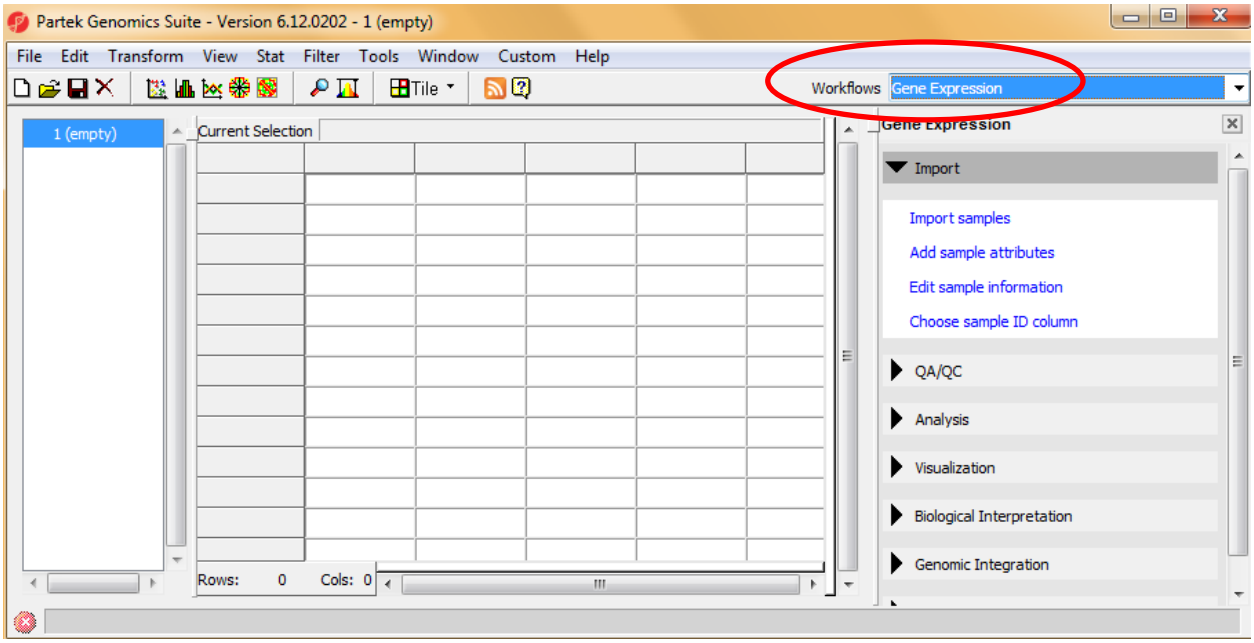


Figure 1: Selecting the gene expression workflow

- Select **Import samples** under the *Import* section of the workflow
- Select **Import from Affymetrix CEL files** and then click **OK**
- Click the **Browse** button to select the *C:\Partek Training Data\Down_Syndrome-GE* folder. By default, all the files with a .CEL extension are selected (Figure 2)

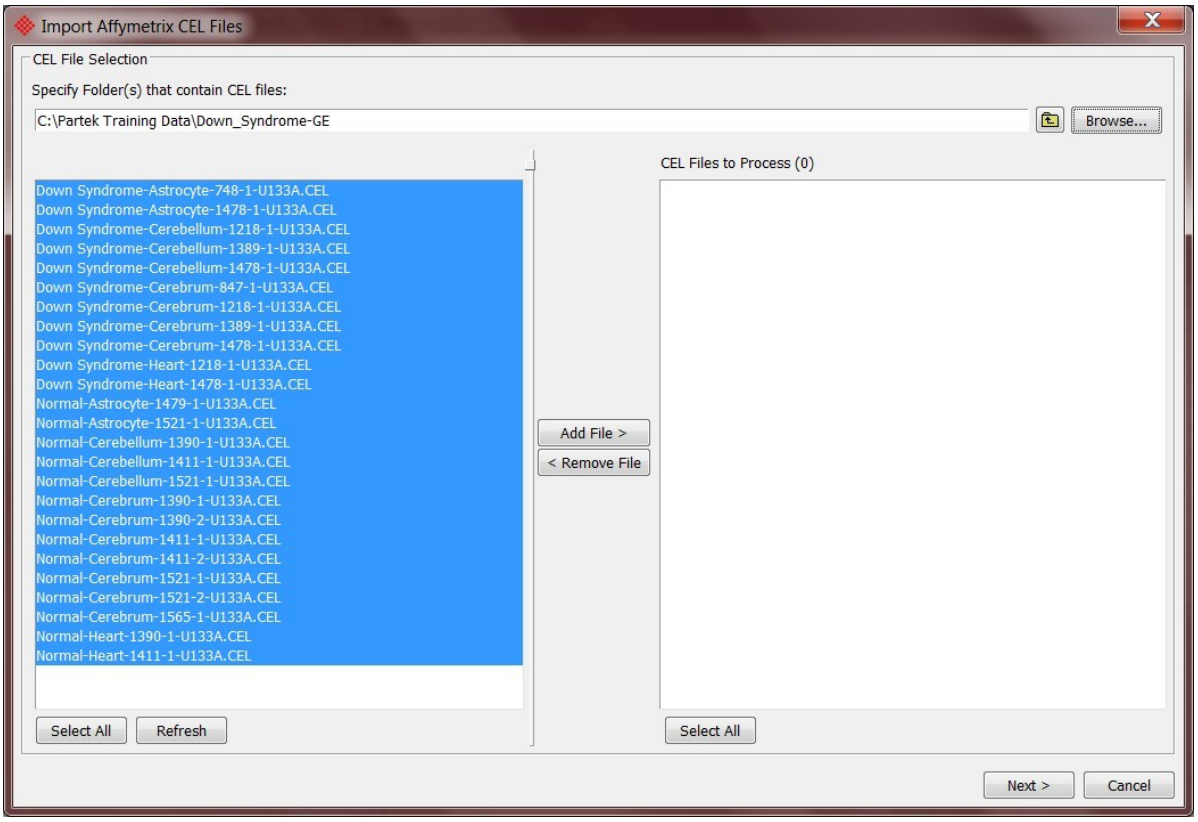


Figure 2: Selecting the folder and CEL files for the experiment

- Select the **Add File >** button to move all the .CEL files to the right panel. Twenty-five CEL files will be processed
- Select **Next;** the *Import Affymetrix CEL Files* dialog is shown in Figure 3

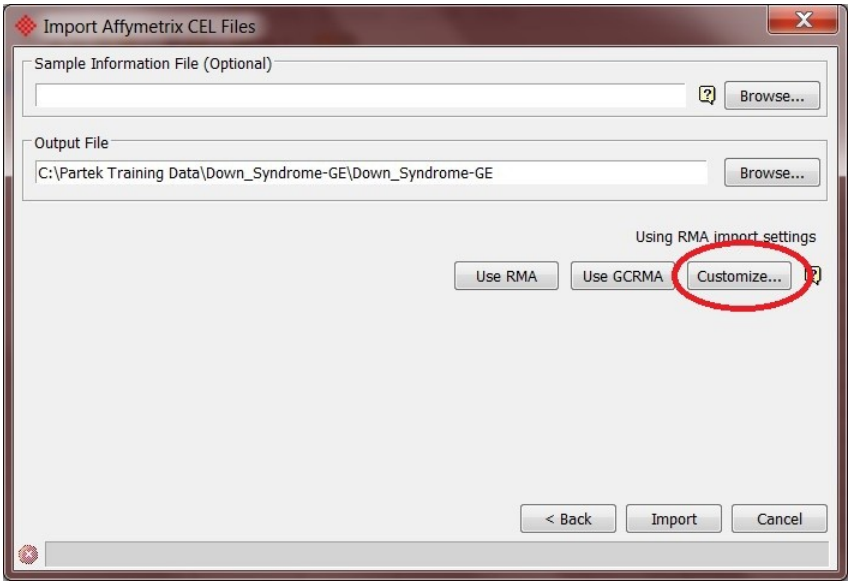


Figure 3: Configuring import files window

- Select **Customize…** to configure the import options (Figure 4)

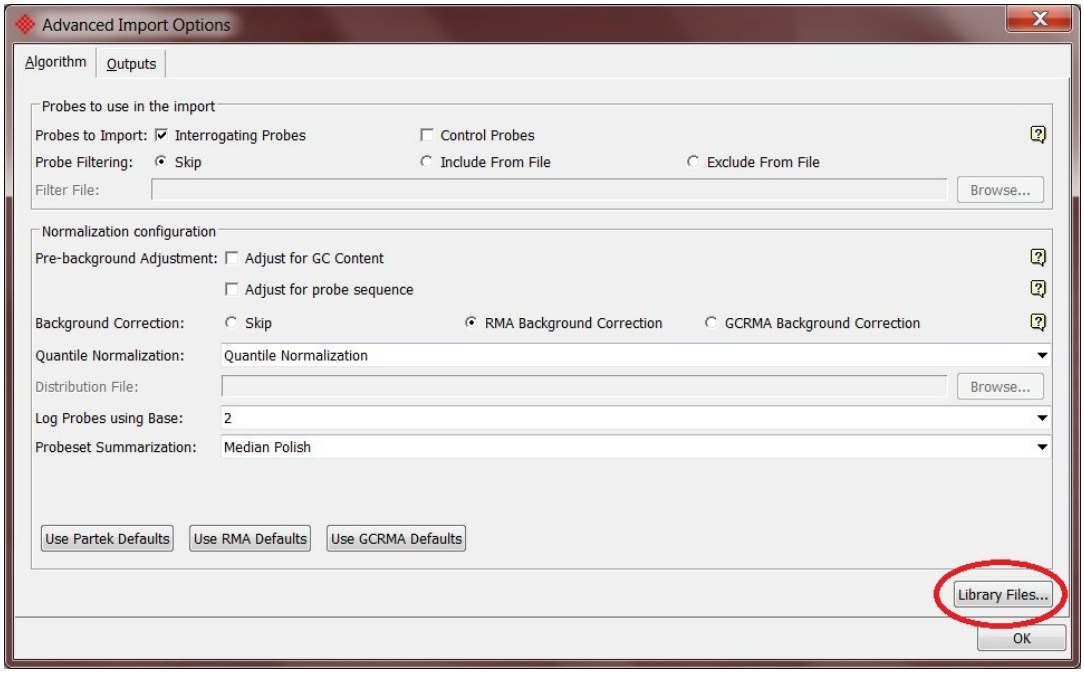


Figure 4: Configuring the Advanced Import Options

- Select "**Library Files…**" to specify the location of the library folder to be used and to specify the annotation files to use (Figure 5)

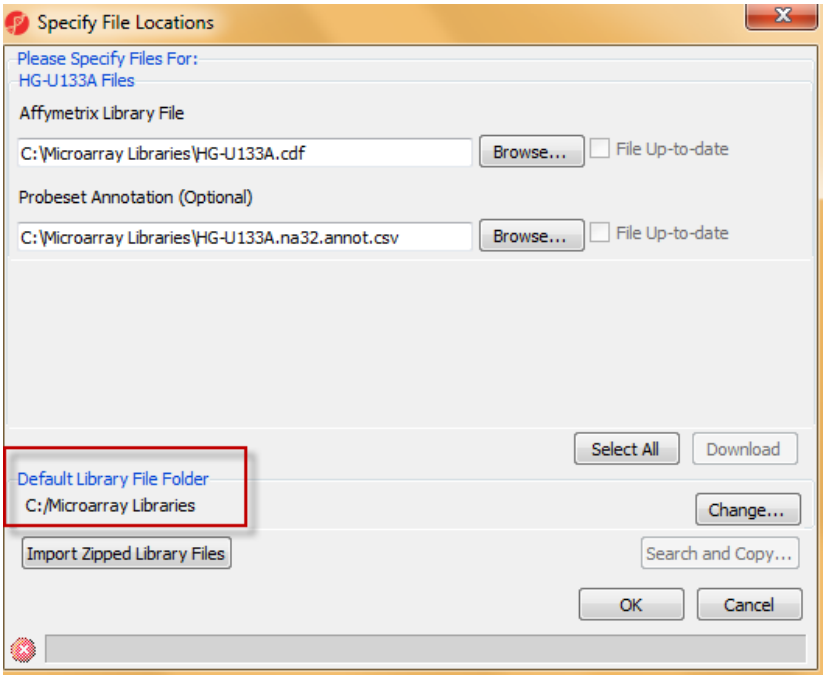


Figure 5: Specifying Microarray Library files or change the default library directory

Partek® Genomics Suite™ will automatically assign the annotation files according to the chip type stored in the .CEL files. If the annotation files are not available in the library directory, Partek® Genomics Suite™ will automatically download them and store them in the Default Library File Folder.

- The default library location can be modified at by selecting the **Change** button in the Default Library File Folder panel. By default, the library directory is at C:\Microarray Libraries. This directory is used to store all the external libraries and annotation files needed for analysis and visualization. The library directory can also be modified from *Tools > File Manager*
- Select **OK** (Figure 5) to close the *Specify File Locations* dialog
- Select the **Outputs** tab from the *Advanced Import Options* dialog (Figure 6)
- Make sure the output chip images based on **Original**, **Summarized,** and **Difference** values are selected
- In the *Extract Time Stamp and Date from CEL File* panel, make sure the **Date** button is selected to extract the chip scan date. This information can help you to detect if there is batch effects caused by the process time
- Select **OK** to exit the *Advanced Import Options* dialog

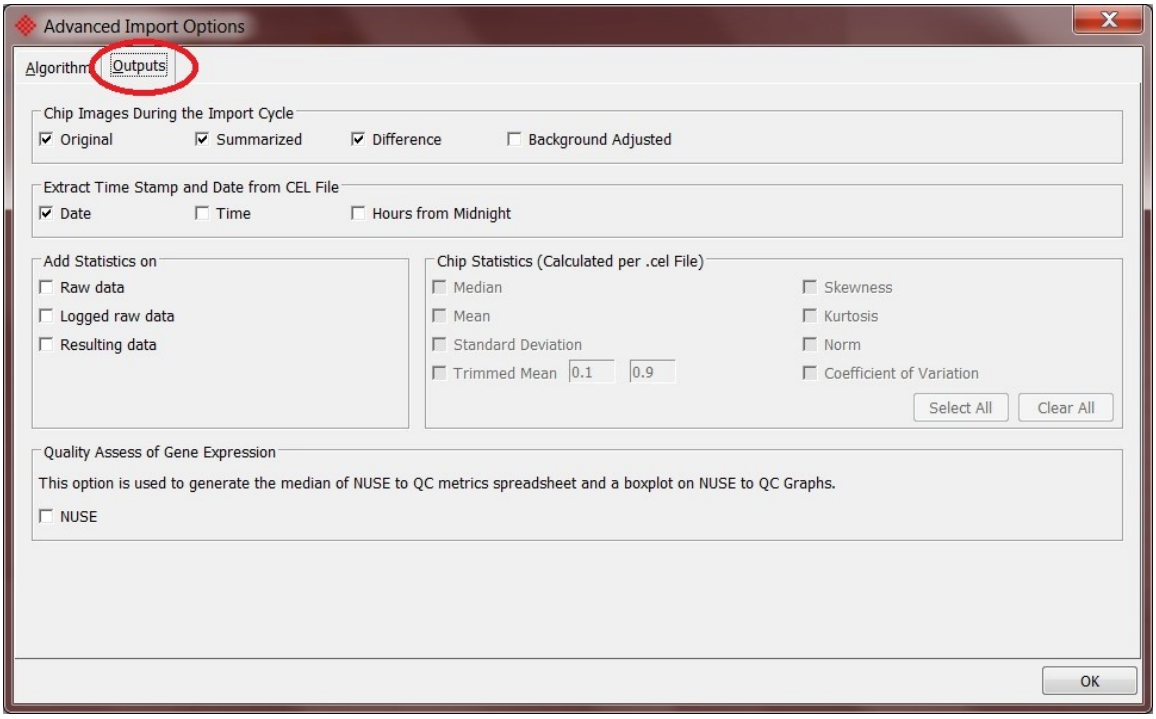


Figure 6: Specifying Advanced Import Options to create chip images of and extract the scan date from the CEL files

- Select **Import** to exit the *Import Affymetrix CEL Files* dialog (Figure 3)

After importing the CEL files has finished, the *postImportQC* spreadsheet, which summarizes intensity values of the Affymetrix control probe-sets, is shown. Also a window with graphical representations of the control probe-sets values appears (Figure 7).

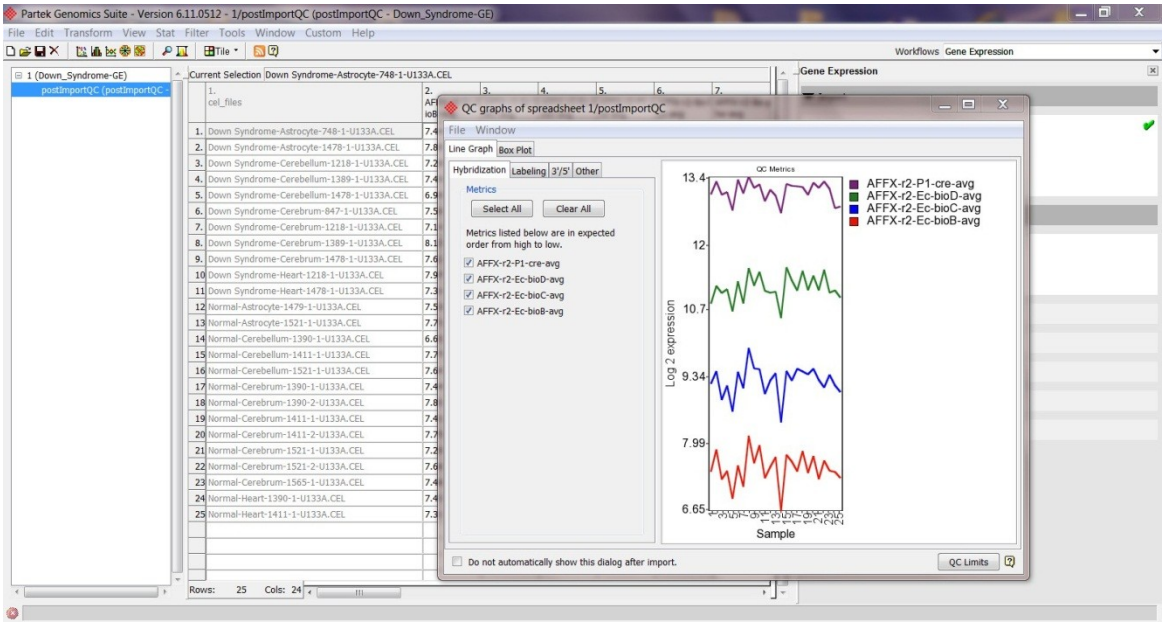


Figure 7: QC metrics are shown in the QC graph as well as in the postImportQC spreadsheet

QC metrics of the *QA/QC* section of the workflow provides quality control information from control and experimental probes on the Affymetrix® chips to provide confidence in the quality of the microarray data or to identify samples that do not meet QC criteria. A more detailed user guide for the Affymetrix QC module is available here or from *Help > On-line Tutorials > User Guides.*

When you close the *QC graphs* window, the result file will be automatically opened in Partek® Genomics Suite™ as *spreadsheet 1 (*named *Down_Syndrome-GE)*. You will see 25 rows representing 25 chips, and more than 22,000 columns representing genes in this spreadsheet (Figure 8).

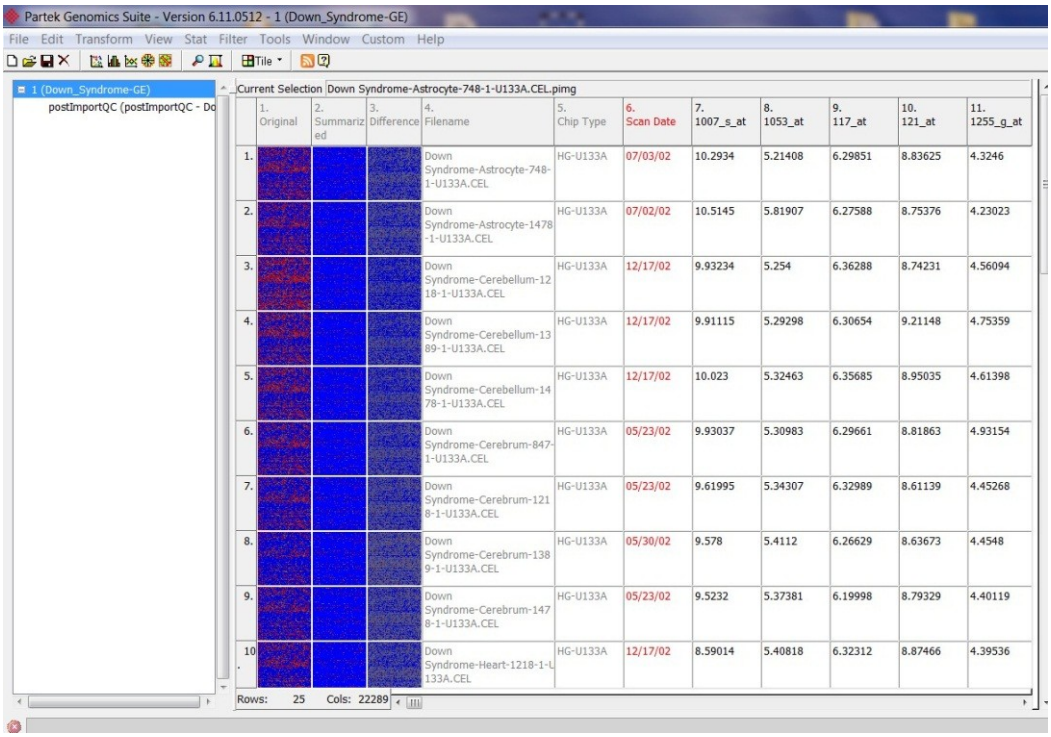

Partek Genomics Suite - Version 6.11.0512 - 1 (Down_Syndrome-GE)
File Edit Transform View Stat Filter Tools Window Custom Help
1 (Down_Syndrome-GE)
postImportQC (postImportQC - Do
Current Selection Down Syndrome-Astrocyte-748-1-U133A.CEL.pimg
1. Original
2. Summarized
3. Difference
4. Filename
5. Chip Type
6. Scan Date
7. 1007_s_at
8. 1053_at
9. 117_at
10. 121_at
11. 1255_g_at
1. Down Syndrome-Astrocyte-748-1-U133A.CEL HG-U133A 07/03/02 10.2934 5.21408 6.29851 8.83625 4.3246
2. Down Syndrome-Astrocyte-1478-1-U133A.CEL HG-U133A 07/02/02 10.5145 5.81907 6.27588 8.75376 4.23023
3. Down Syndrome-Cerebellum-1218-1-U133A.CEL HG-U133A 12/17/02 9.93234 5.254 6.36288 8.74231 4.56094
4. Down Syndrome-Cerebellum-1389-1-U133A.CEL HG-U133A 12/17/02 9.91115 5.29298 6.30654 9.21148 4.75359
5. Down Syndrome-Cerebellum-1478-1-U133A.CEL HG-U133A 12/17/02 10.023 5.32463 6.35685 8.95035 4.61398
6. Down Syndrome-Cerebrum-847-1-U133A.CEL HG-U133A 05/23/02 9.93037 5.30983 6.29661 8.81863 4.93154
7. Down Syndrome-Cerebrum-1218-1-U133A.CEL HG-U133A 05/23/02 9.61995 5.34307 6.32989 8.61139 4.45268
8. Down Syndrome-Cerebrum-1389-1-U133A.CEL HG-U133A 05/30/02 9.578 5.4112 6.26629 8.63673 4.4548
9. Down Syndrome-Cerebrum-1478-1-U133A.CEL HG-U133A 05/23/02 9.5232 5.37381 6.19998 8.79329 4.40119
10. Down Syndrome-Heart-1218-1-U133A.CEL HG-U133A 12/17/02 8.59014 5.40818 6.32312 8.87466 4.39536
Rows: 25 Cols: 22289

Figure 8: Viewing the main or top-level spreadsheet with .CEL file images

- Double click on any of the chip image thumbnails to enlarge the image and to examine the chip (Figure 9)

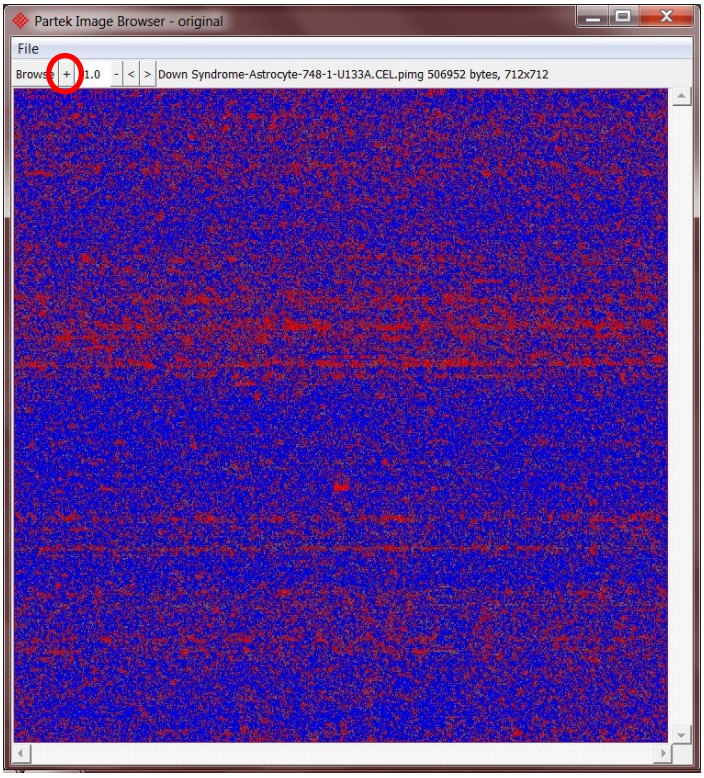


Figure 9: Viewing a chip image

- Click on the + button to zoom in on the image; click on the – button to zoom out. The scroll bars on the right and across the bottom of the window may be needed to navigate around the image at higher zoom levels

These pseudo chip images may be used to identify any anomalies with the chip such as scratches, hybridization errors, and scanning errors if you suspect there might be a problem with the chip. This check is usually performed on outliers in the QA/QC step of the gene expression workflow.

For additional information on importing data into Partek® Genomics Suite™, see **Chapter 4 Importing and Exporting Data** in the Partek User's Manual. The User's Manual is available from the Partek Genomic Suite menu from *Help> User's Manual.* The FAQ (*Help > On-line Tutorials > FAQ*) may also be helpful. As this tutorial only addresses some topics, you may need to consult the User's Manual for additional information about other useful features.

It is recommended that you are familiar with **Chapter 6 The Pattern Visualization System®** of the user manual before going through the next section of the tutorial.

Adding Sample Information

Twenty-five CEL files (samples) have been imported into Partek® Genomics Suite™ as shown in Figure 8. Sample information must be added in order to define the grouping and the goals of the experiment.

- Select **Add sample attributes** in the *Import* section of the workflow
- Choose the option **Add attributes from an existing column** as shown in Figure 10 and select **OK**

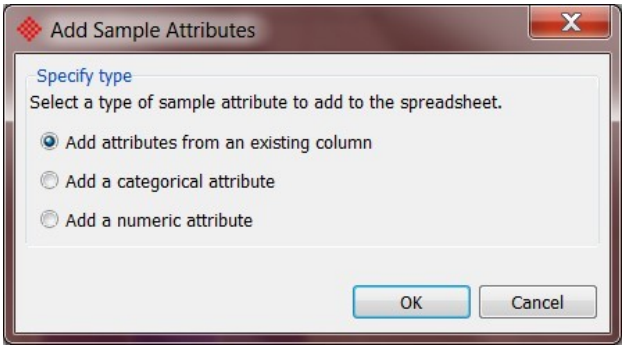

Add Sample Attributes
Specify type
Select a type of sample attribute to add to the spreadsheet.
Add attributes from an existing column
Add a categorical attribute
Add a numeric attribute
OK
Cancel

Figure 10: Configuring the Add Sample Attributes dialog

In this tutorial, the file name (e.g., Down Syndrome-Astrocyte-748-1-U133A.CEL) contains the information about a particular sample and is separated by hyphens (-). Choosing to split the file name by delimiters will separate the categories into different columns as shown in Figure 11.

- In the *Sample Information* panel, specify the column labels (*Labels 1-4*) as **Type**, **Tissue,** and **Subject**, respectively, as categorical and **skip** the other columns as shown in Figure 11. Select **OK**

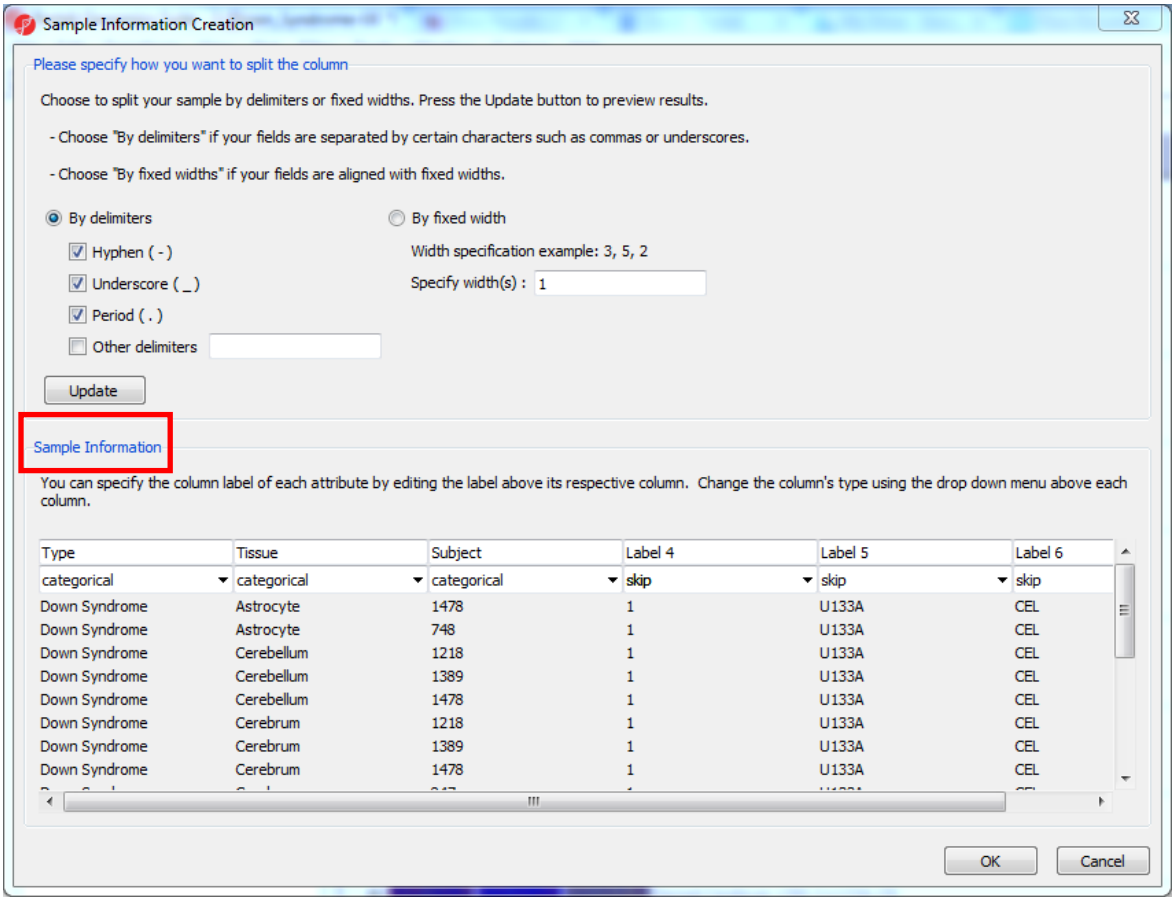
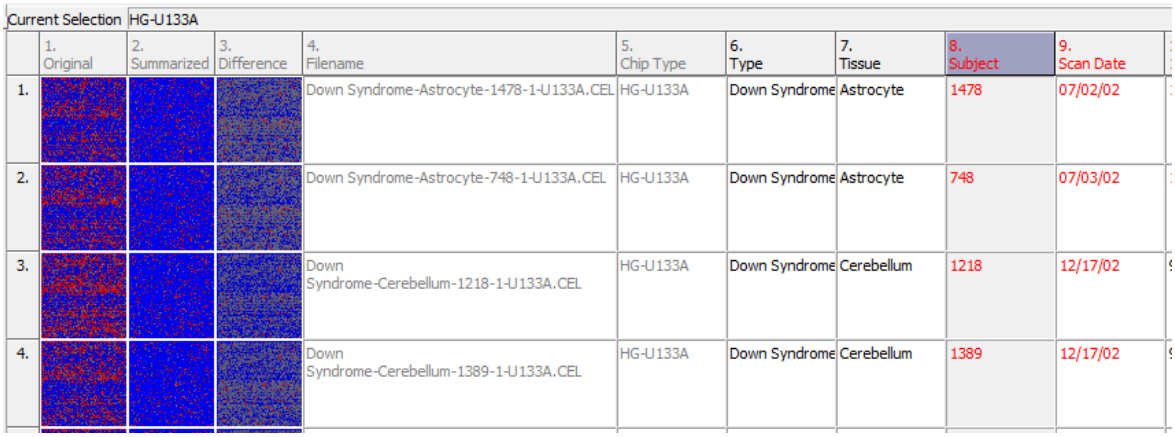

Sample Information Creation
Please specify how you want to split the column
Choose to split your sample by delimiters or fixed widths. Press the Update button to preview results.
- Choose "By delimiters" if your fields are separated by certain characters such as commas or underscores.
- Choose "By fixed widths" if your fields are aligned with fixed widths.
By delimiters
Hyphen (-)
Underscore (_)
Period (.)
Other delimiters
By fixed width
Width specification example: 3, 5, 2
Specify width(s) : 1
Update
Sample Information
You can specify the column label of each attribute by editing the label above its respective column. Change the column's type using the drop down menu above each column.
Type Tissue Subject Label 4 Label 5 Label 6
categorical categorical categorical skip skip skip
Down Syndrome Astrocyte 1478 1 U133A CEL
Down Syndrome Astrocyte 748 1 U133A CEL
Down Syndrome Cerebellum 1218 1 U133A CEL
Down Syndrome Cerebellum 1389 1 U133A CEL
Down Syndrome Cerebellum 1478 1 U133A CEL
Down Syndrome Cerebrum 1218 1 U133A CEL
Down Syndrome Cerebrum 1389 1 U133A CEL
Down Syndrome Cerebrum 1478 1 U133A CEL
OK
Cancel

Figure 11: Configuring the Sample Information Creation dialog

- A dialog window asking if you would like to save the spreadsheet will appear. Select **Yes** to save the spreadsheet with new sample attributes
- Make column 8 (**Subject)** random by right-clicking on the column header and selecting **Properties**. Select the **Random Effect** check box**.** The spreadsheet containing the added sample information is as shown Figure 12

Note: More details on *Random vs. Fixed Effects* can be found later in this tutorial under the section **Identifying Differentially Expressed Genes using the ANOVA**.



Current Selection: HG-U133A

	1. Original	2. Summarized	3. Difference	4. Filename	5. Chip Type	6. Type	7. Tissue	8. Subject	9. Scan Date
1.				Down Syndrome-Astrocyte-1478-1-U133A.CEL	HG-U133A	Down Syndrome	Astrocyte	1478	07/02/02
2.				Down Syndrome-Astrocyte-748-1-U133A.CEL	HG-U133A	Down Syndrome	Astrocyte	748	07/03/02
3.				Down Syndrome-Cerebellum-1218-1-U133A.CEL	HG-U133A	Down Syndrome	Cerebellum	1218	12/17/02
4.				Down Syndrome-Cerebellum-1389-1-U133A.CEL	HG-U133A	Down Syndrome	Cerebellum	1389	12/17/02

Figure 12: Viewing the spreadsheet with added sample information

Exploratory Data Analysis

At this point in analysis, you would explore the data preliminarily. Do the genes you expected to be differentially regulated appear to have larger or smaller intensity values? Do similar samples resemble each other?

The latter question can be explored using Principal Components Analysis (PCA), an excellent method for reducing and visualizing high-dimensional data.

- Select **Principal Components Analysis (PCA)** in *QA/QC* section of the *Workflows* dialog. Select **6. Type** from the panel on the right side. The *Scatter Plot* dialog box with your PCA plot will appear as shown in Figure 13

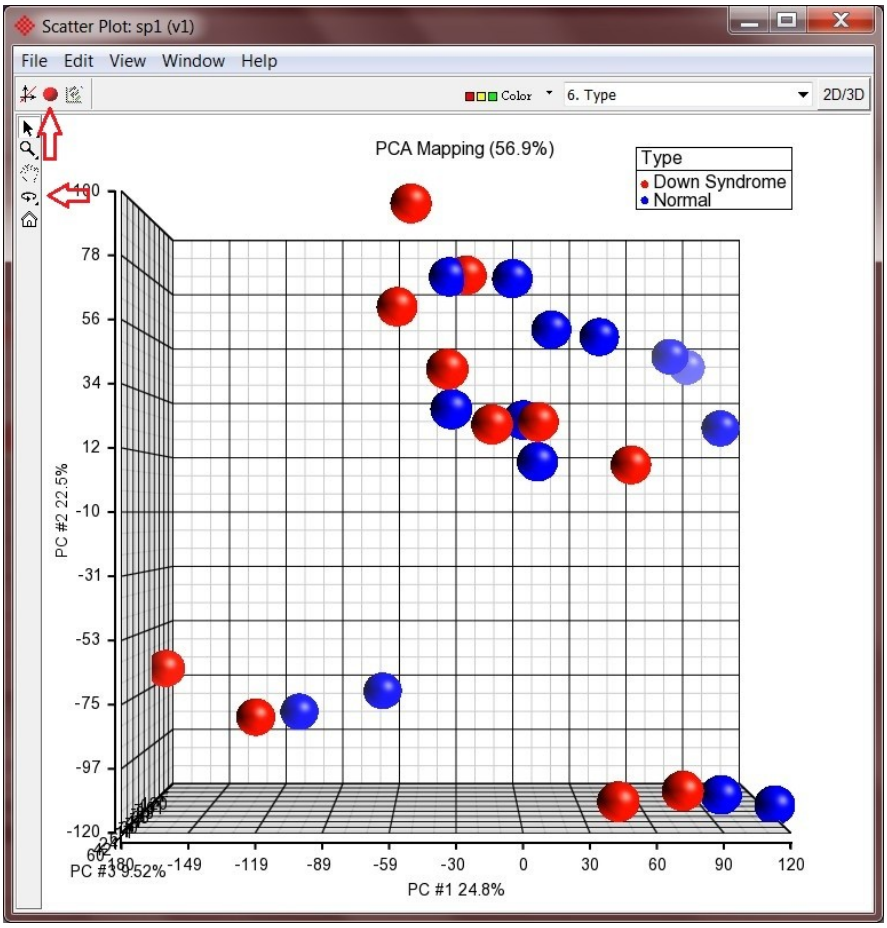


Figure 13: Viewing the PCA scatter plot of the Down syndrome data. Each dot represents a chip; the color of the dot represents the Type (Down's or normal) of the sample

In the scatter plot, each point represents a chip (sample) and corresponds to a row on the top-level spreadsheet. The color of the dot represents the type of the sample; red represents a Down syndrome sample and blue represents a normal sample. Points that are close together in the plot have similar intensity values across the probesets on the whole chip (genome), and points that are far apart in the plot are dissimilar.

- Left-click on any point in the scatter plot, and the corresponding row will be highlighted in the spreadsheet
- While pressing the mouse wheel down, drag the mouse to rotate the plot or choose the ***Rotate Mode*** option ( ) on the left side of the *Scatter Plot* window. Press and drag the left mouse button to rotate the plot to examine the grouping pattern or outliers of the data on the first 3 principal components (PCs)
- Scrolling the mouse wheel up or down will zoom in and out

As you can see from rotating the plot, there is no clear separation between Down syndrome and normal samples in this data since the red and blue samples are not separated in space. However, there are other factors that may separate the data.

- In the *Scatter Plot* viewer, select the *Plot Properties* icon ( ) and configure the plot as shown in Figure 14.
- *Color* the points by column **7**. **Tissue** and *Size* the points by column **6. Type**
- Select **Apply**

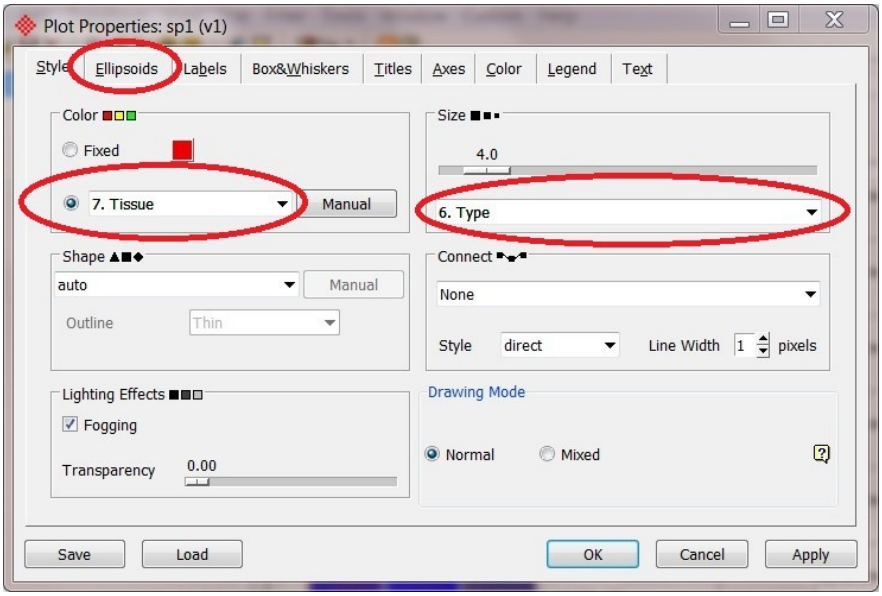


Figure 14: Configuring the PCA scatter plot: Color by Tissue, Size by Type

Notice now that the data are clustered by different tissues (Figure 15).

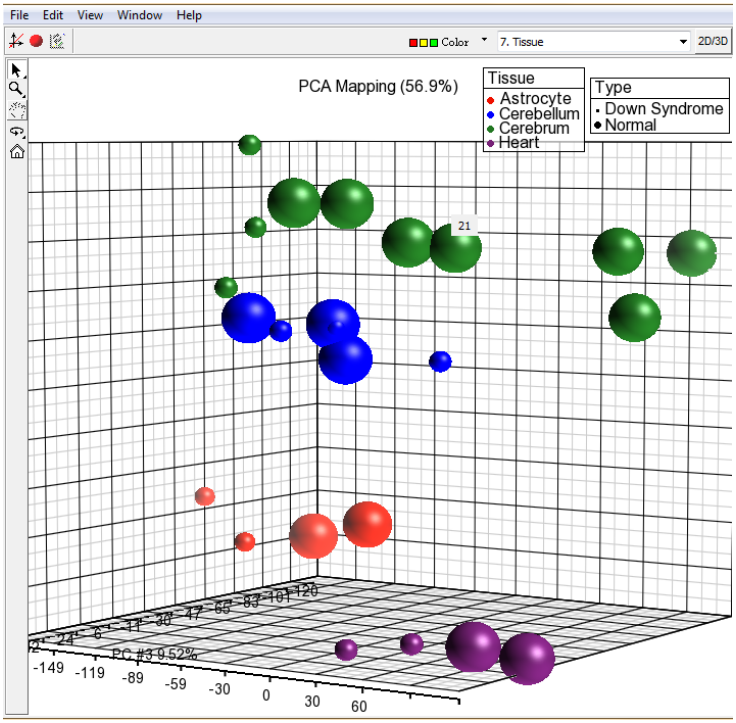


Figure 15: Down syndrome data colored by Tissue and sized by Type

- Another way to see the cluster pattern is to put an ellipse around the *Tissue* groups. Select the **Ellipsoids** tab on the *Plot Properties* dialog
- Select **Add Ellipse/Ellipsoid**
- Select the **Ellipse** radio button
- Double click on **Tissue** to move it to the *Grouping Variable(s)* panel
- Select **OK (**Figure 16**)** to exit the *Add Ellipse/Ellipsoid* dialog and **OK** again to add the ellipse

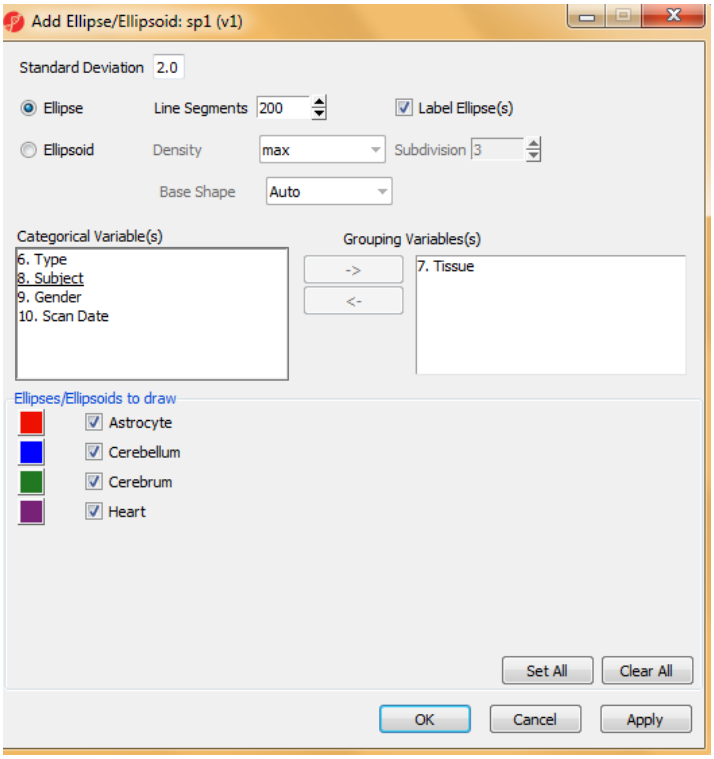


Figure 16: Adding an Ellipse on Tissue

By rotating this plot, you can see that the data is separated by tissues, and within some of the tissues, the Down's samples and normal samples are separated. For instance in the *Astrocyte* and *Heart* tissues, the Down syndrome samples (small dots) are on the left, and the normal samples (large dots) are on the right (Figure 17).

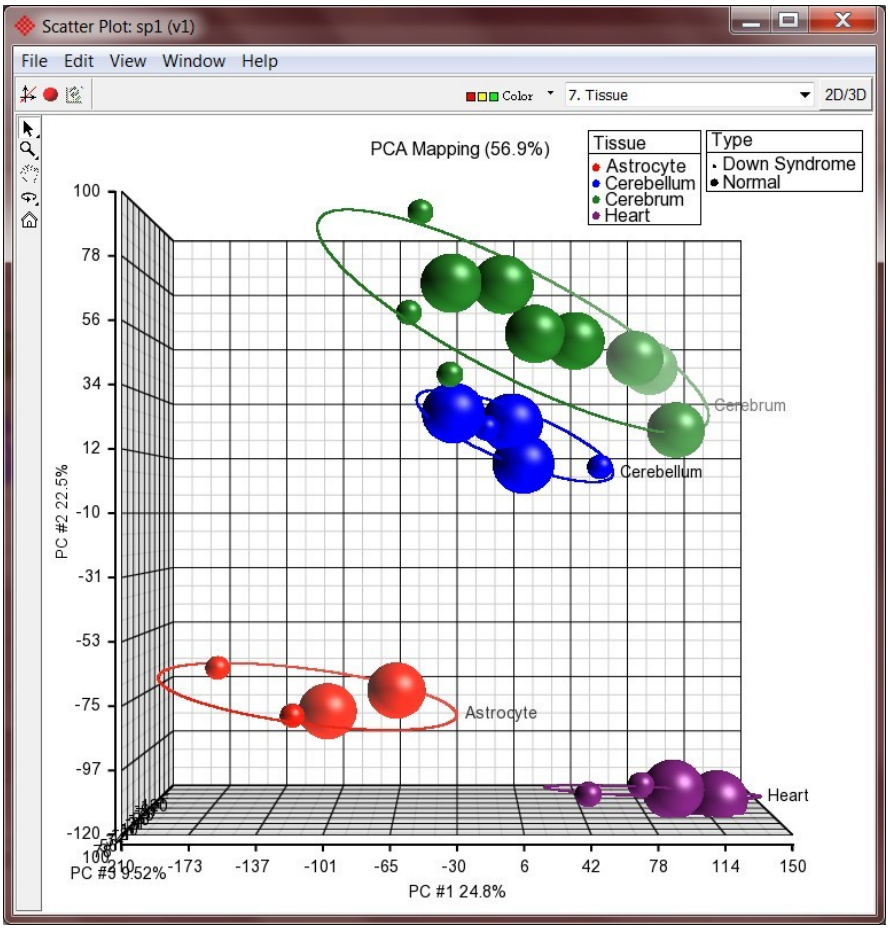


Figure 17: Viewing a scatter plot of data colored by Tissue, sized by Type, and grouped by Tissue

PCA is an example of exploratory data analysis and is useful for identifying outliers and major effects in the data. From the scatter plot, you can see that the tissue is the biggest source of variation. There are many genes that express differently between the 4 tissues, but not as many genes that express differently between type (Down syndrome and normal) across the whole chip (genome).

The next step in the workflow is to draw a histogram to examine the samples. Select **Plot sample histogram** in the *QA/QC* section of the *Workflow* to get the *Histogram* dialog box as shown in Figure 18.

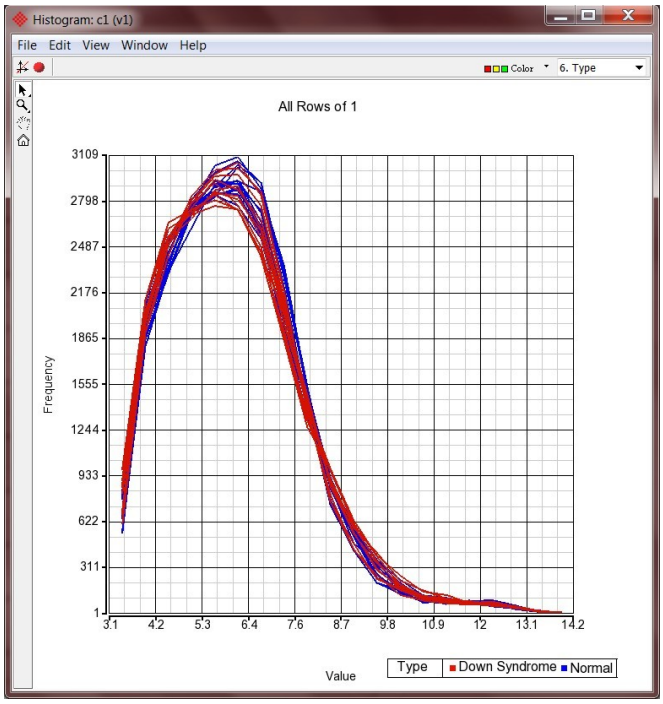


Figure 18: Viewing the histogram of all 25 samples

The histogram plots one line for each of the samples with the intensity of the probes graphed on the X-axis and the frequency of the probe intensity on the Y-axis. This allows you to view the distribution of the intensities to identify any outliers. In this dataset, all of the samples follow the same distribution pattern indicating that there are no obvious outliers in the data. As demonstrated with the PCA, if you click on any of the lines in the histogram, the corresponding row will be highlighted in the spreadsheet *Down_Syndrome-GE*. You can also change the way the histogram displays the data by clicking on the *Plot Properties* button. Explore these options on your own.

The last option in the **QA/QC** section is **QC metrics** which has already been discussed.

The decision to discard any samples would be based on information from the PCA plot, pseudo chip images, sample histogram plot, and QC metrics. To discard a sample and renormalize the data (without the effects of the outlier), start over with importing samples and omit the outlier sample(s) during the CEL file import.

Identifying Differentially Expressed Genes using the ANOVA

Analysis of variance (ANOVA) is a very powerful technique for identifying differentially expressed genes in a multi-factor experiment such as this one. In this data set, the ANOVA will be used to generate a list of genes that are significantly different between Down syndrome and normal with an absolute difference bigger than 1.3 fold.

The ANOVA model should include *Type* since it is the primary factor of interest. From the exploratory analysis, tissue was found to be a large source of variation; therefore, tissue

should be included in the model. In the experiment, multiple samples were taken from the same subject, so *Subject* must be included in the model; otherwise, the ANOVA assumption that samples within groups are independent will be violated. In addition, the PCA scatter plot showed that the Down's and normal separated within tissue type, so the Type*Tissue interaction should be included in the model.

- To invoke the ANOVA dialog, click **Detect differentially expressed genes** in the *Analysis* section of the workflow
- In the *Experimental Factor(s)* panel, select **Type, Tissue** and **Subject** by pressing <Ctrl> and left clicking
- Use the **Add Factor >** button to move the selections to the *ANOVA Factor(s)* panel
- To specify the interaction, select **Type** and **Tissue** by pressing <Ctrl> and left clicking. Select the **Add Interaction >** button to add the **Type * Tissue** interaction in the *ANOVA Factor(s)* panel (Figure 19). Do NOT select **OK**

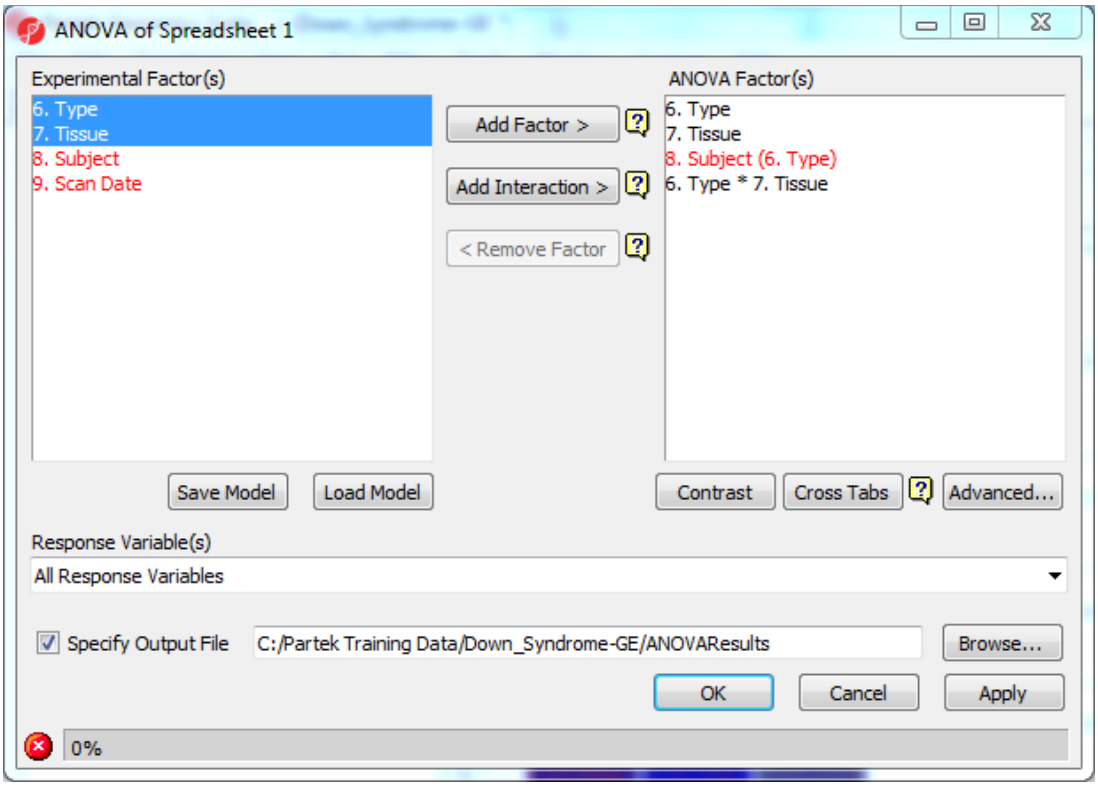


Figure 19: Factors and interactions to be included in the ANOVA model

Random vs. Fixed Effects – Mixed Model ANOVA

Most factors in analysis of variance (ANOVA) are fixed effects, whose levels represent all the levels of interest. In this study, *Type* and *Tissue* are fixed effects. If the levels of a factor only represent a random sample of all the levels of interest (for instance, *Subject*), the factor is a random effect. The ten subjects in this study represent only a random sample of the global population about which inferences are being made. Random effects appear in red

on the spreadsheet and in the ANOVA dialog. When the ANOVA model includes both random and fixed factors, it is a mixed-model ANOVA.

Here is another way to tell if a factor is random or fixed: imagine repeating the experiment. Would the same levels of each factor be used again?

- Type – Yes, the same types would be used again - a **fixed** effect
- Tissue – Yes, the same tissues would be used again - a **fixed** effect
- Subject - No, the samples would be taken from other subjects- a **random** effect

You can specify which factors are random and which are fixed when you import your data or after importing by right-clicking on the column corresponding to a categorical variable, selecting **Properties,** and checking **Random effect**. By doing that, the ANOVA will automatically know which factors to treat as random and which factors to treat as fixed.

Nested/Nesting Relationships

The subject factor in the ANOVA model is listed as "Subject (Type)" this means that *Subject* is nested in *Type*. Partek® Genomics Suite™ can automatically detect this sort of hierarchical design and will make adjustments to the ANOVA calculation accordingly.

Linear Contrasts

By default, an ANOVA only outputs a p-value for each factor/interaction; therefore, to get the fold change and ratio between Down syndrome and normal, a contrast must be set-up.

- Select the **Contrast** button (Figure 19) to invoke the *Configure* dialog
- Choose **6. Type** from the *Select Factor/Interaction* drop-down list. All of the levels in this factor are listed on the *Candidate Level(s)* panel on the left of the dialog (Figure 20)
- Select *Down Syndrome* from the *Candidate Level(s)* panel and move it to the **Down Syndrome (***Group 1)* panel by selecting **Add Contrast Level >** in the top half of the dialog. *Label 1* will be changed to the subgroup name automatically, but you can also manually specify the label name
- Select *Normal* from the *Candidate Level(s)* panel and move it to the **Normal (***Group 2)* panel in a similar manner

Since the data is $\log_2$ transformed, Partek® Genomics Suite™ will automatically detect this and will automatically select the radio button *Yes* in the *Data is already log transformed?* at the top right hand corner. Partek® Genomics Suite™ will use the geometric mean of the samples in each group to calculate the fold change and mean ratio for the contrast between the *Down syndrome* and *Normal* samples.

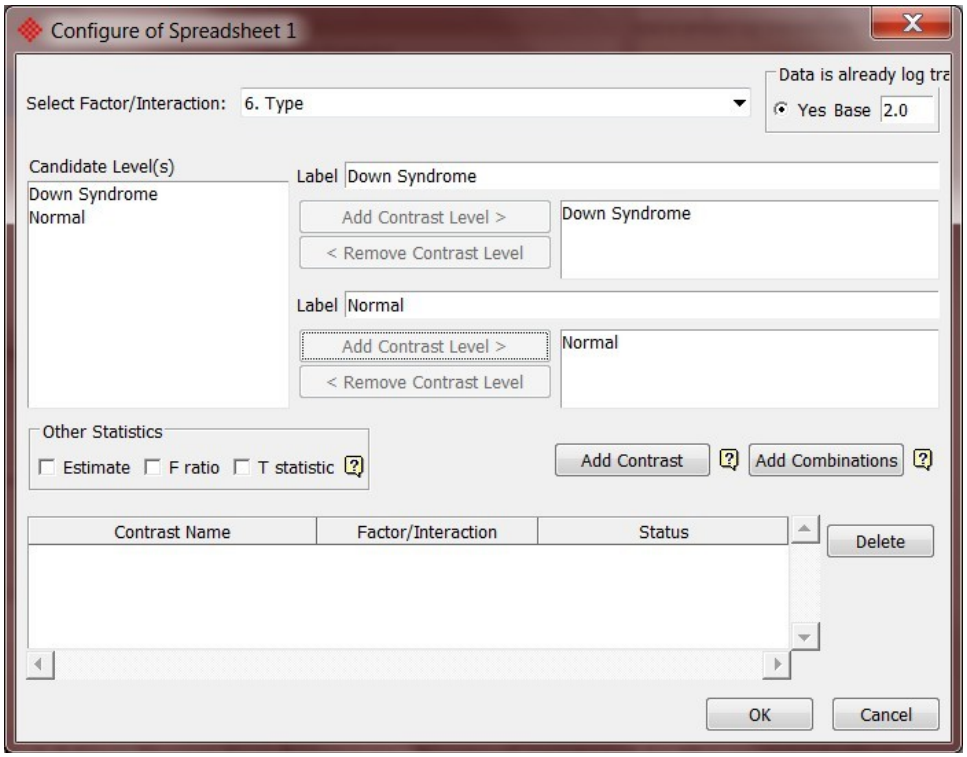


Figure 20: Adding Down Syndrome vs. Normal contrast to the computation

- Select **Add Contrast** to add the *Down Syndrome vs. Normal* contrast (Figure 21)
- Select **OK** to apply the configuration

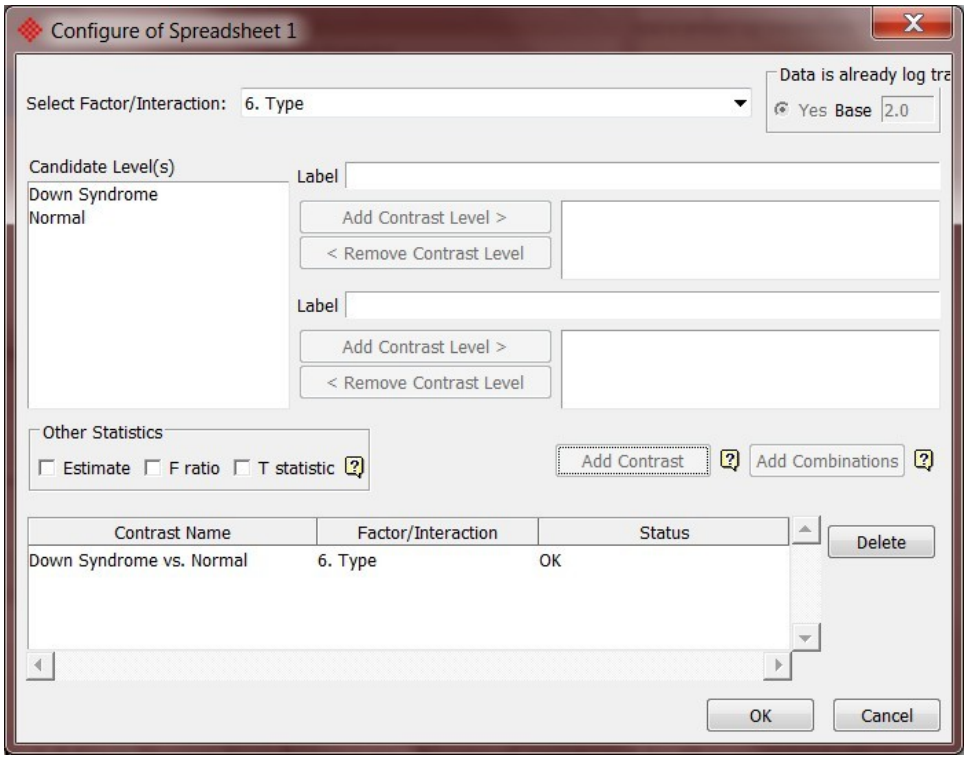


Figure 21: Configuring the Adding Contrasts dialog

- By default the *Specify Output File* is checked in Figure 19 and gives a name to the output file. If you are trying to determine which factors should be included in the model and you do not wish to save the output file, simply uncheck this box
- Select **OK** or **Apply** in the *ANOVA* dialog to compute the 3-way mixed-model ANOVA

The result will be displayed in a child spreadsheet, *ANOVA-3way(ANOVAResults)*. In the child result spreadsheet, each row represents a gene, and the columns represent the computation results for that gene (Figure 22). By default, the genes are sorted in ascending order by the p-value of the first categorical factor, *Type*, which means the most significant differently expressed gene between Down syndrome and normal is at the top of the spreadsheet.

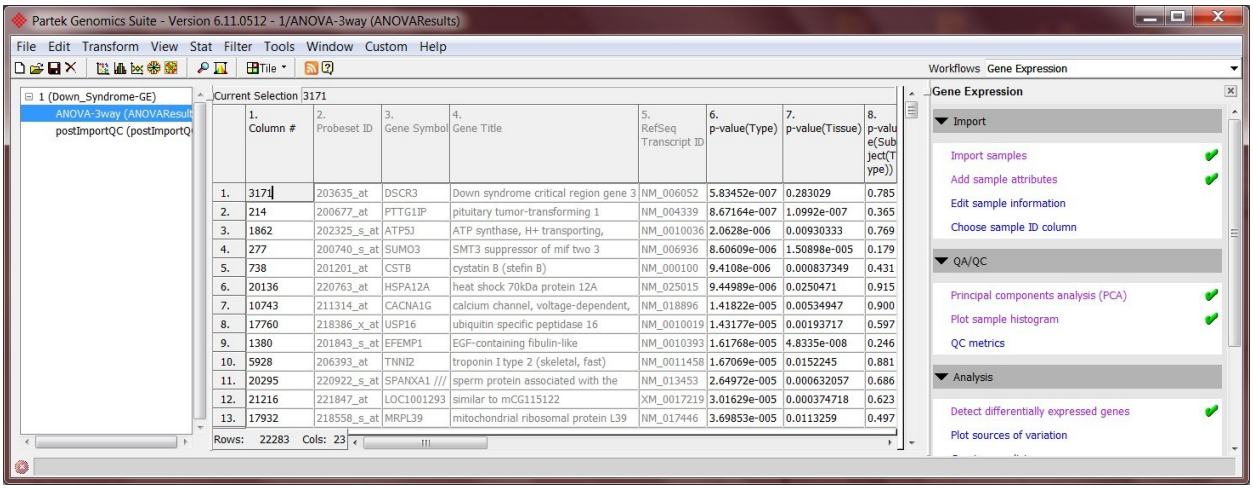

Partek Genomics Suite - Version 6.11.0512 - 1/ANOVA-3way (ANOVAResults)
File Edit Transform View Stat Filter Tools Window Custom Help
Workflows Gene Expression
1 (Down_Syndrome-GE)
ANOVA-3way (ANOVAResult
postImportQC (postImportQ
Current Selection 3171
1. Column #
2. Probeset ID
3. Gene Symbol
4. Gene Title
5. RefSeq Transcript ID
6. p-value(Type)
7. p-value(Tissue)
8. p-value(Subject(Type))
1. 3171 203635_at DSCR3 Down syndrome critical region gene 3 NM_006052 5.83452e-007 0.283029 0.785
2. 214 200677_at PTTG1IP pituitary tumor-transforming 1 NM_004339 8.67164e-007 1.0992e-007 0.365
3. 1862 202325_s_at ATP5J ATP synthase, H+ transporting, NM_0010036 2.0628e-006 0.00930333 0.769
4. 277 200740_s_at SUMO3 SMT3 suppressor of mif two 3 NM_006936 8.60609e-006 1.50898e-005 0.179
5. 738 201201_at CSTB cystatin B (stefin B) NM_000100 9.4108e-006 0.000837349 0.431
6. 20136 220763_at HSPA12A heat shock 70kDa protein 12A NM_025015 9.44989e-006 0.0250471 0.915
7. 10743 211314_at CACNA1G calcium channel, voltage-dependent, NM_018896 1.41822e-005 0.00534947 0.900
8. 17760 218386_x_at USP16 ubiquitin specific peptidase 16 NM_0010019 1.43177e-005 0.00193717 0.597
9. 1380 201843_s_at EFEMP1 EGF-containing fibulin-like NM_0010393 1.61768e-005 4.8335e-008 0.246
10. 5928 206393_at TNNI2 troponin I type 2 (skeletal, fast) NM_0011458 1.67069e-005 0.0152245 0.881
11. 20295 220922_s_at SPANXA1 /// sperm protein associated with the NM_013453 2.64972e-005 0.000632057 0.686
12. 21216 221847_at LOC1001293 similar to mCG115122 XM_0017219 3.01629e-005 0.000374718 0.623
13. 17932 218558_s_at MRPL39 mitochondrial ribosomal protein L39 NM_017446 3.69853e-005 0.0113259 0.497
Rows: 22283 Cols: 23
Gene Expression
Import
Import samples
Add sample attributes
Edit sample information
Choose sample ID column
QA/QC
Principal components analysis (PCA)
Plot sample histogram
QC metrics
Analysis
Detect differentially expressed genes
Plot sources of variation

Figure 22: Viewing the ANOVA results (child spreadsheet)

For additional information about ANOVA in Partek® Genomics Suite™, see **Chapter 11 Inferential Statistics** in the User's Manual (*Help > User's Manual*).

Viewing the Sources of Variation

Deciding which factors to include in the ANOVA may be an iterative process while you decide which factors and interactions are relevant as not all factors have to be included in the model. For instance, in this example, *Gender* and *Scan date* were not included. The *Sources of Variation* plot is a way to quantify the relative contribution of each factor in the model in explaining the variability of the data.

- View the sources of variation for each of the factors across the whole genome by clicking **Plot sources of variation** from the *Analysis* section of the workflow with the ANOVA result spreadsheet active
- A *Sources of Variation* Plot dialog box will appear. You have a choice of viewing the sources of variation as a bar chart (Signal to Noise Ratio) or as a pie chart (Sum of Squares)
- Select the **Bar Chart** tab and **Apply**. The *Sources of Variation* plot is shown in (Figure 23)

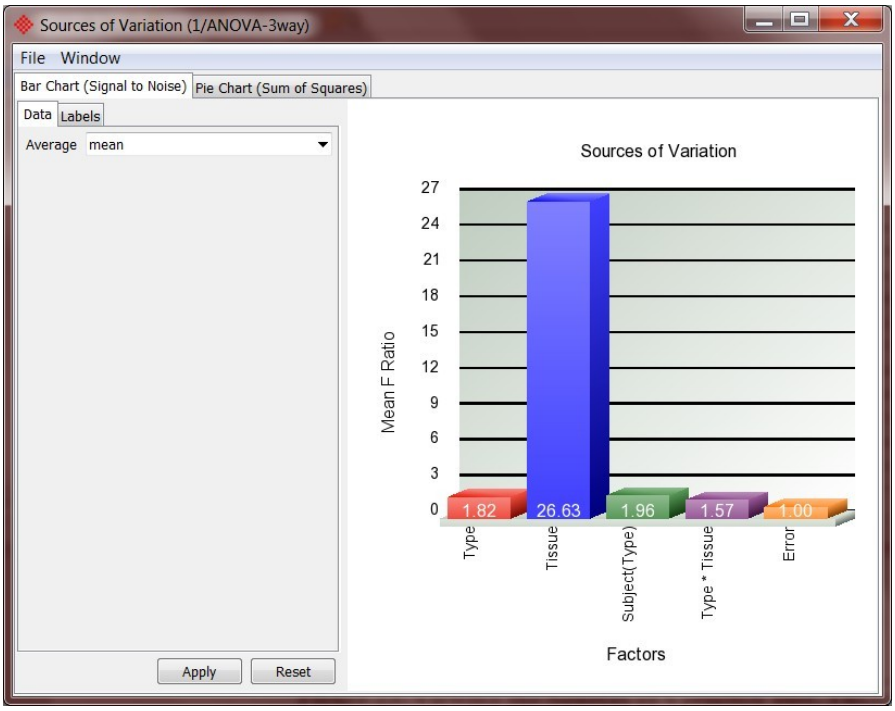


Figure 23: Viewing the Sources of Variation plot which shows Tissue is the biggest source of variation overall

This plot presents the mean signal-to- noise ratio of all the genes on the microarray. All the factors in the ANOVA model are listed on the X-axis (including random error). The Y-axis represents the mean of the ratios of mean square of all the genes to the mean square error of all the genes. Mean square is ANOVA's measure of variance. Compare each signal bar to the error bar; if a bar is higher than the error bar, it means that factor contributed significant variation to the data across all the variables. Notice, that this plot is very consistent with the results in the PCA scatter plot. In this data, on average, *Tissue* is the biggest source of variation.

To view the source of variation of each individual gene, right click on a row header in the ANOVA spreadsheet and select the **Sources of Variation** item from the pop-up menu. View a few plots from rows at the top of the ANOVA table and some from the bottom of the table.

Another useful graph is the *ANOVA Interaction Plot* which is also accessed by right-clicking on a row header in the ANOVA spreadsheet. Select **ANOVA Interaction Plot** from the options. Generate these plots for rows 5 (CSTB) and row 7 (CACNA1G). If the lines in this plot are not parallel, then there is a chance there is an interaction between *Tissue* and *Type*. Look at the p-values in column 9, *p-value(Type * Tissue)*.

Create Gene List

Now that you have obtained statistical results from the microarray experiment, you can now take the result of 22,283 genes and create a new spreadsheet of just those genes that pass a certain criteria. This will make managing the data more streamlined by focusing on

just those genes with the most significant differential expression or substantial fold change. In Partek® Genomics Suite™, the *List Manager* can be used to specify numerous conditions to use in the generation of our list of genes of interest. In this tutorial, we are going to create a gene list with a fold change between –1.3 to 1.3 with the significance FDR of 10%. The following section will illustrate how to use the *List Manager* to create this gene list.

- To invoke the *List Creator*, select **Create gene list** in the *Analysis* section of the workflow
- Ensure that the *1/ANOVA-3way (ANOVAResults)* spreadsheet is selected as this is the spreadsheet we will be using to create our new gene list as shown in Figure 24
- Select the **ANOVA Streamlined** tab. In the *Contrast* panel, choose **Down Syndrome vs. Normal** in the name and **Have Any Change** from the *Setting* dropdown menu list. This will find genes that have a fold change different between the different types of samples
- In the *Configuration for "Down Syndrome vs Normal"* panel, check the *Include size of the change* and enter *Fold change* > **1.3** *OR Fold change* < **–1.3**
- Select *Include significance of the change* **p-value with FDR < 0.1**. The number of genes that pass your cut off criteria will be shown next to the # *Pass* field. In this example, 16 genes pass the criteria of FDR with 0.10. That is, 10% of the genes in that list are expected to be "false positives," that is, do not demonstrate significant fold change of less than -1.3 or greater than 1.3
- *Save the list* as **A,** select the **Create** button, and **Close**

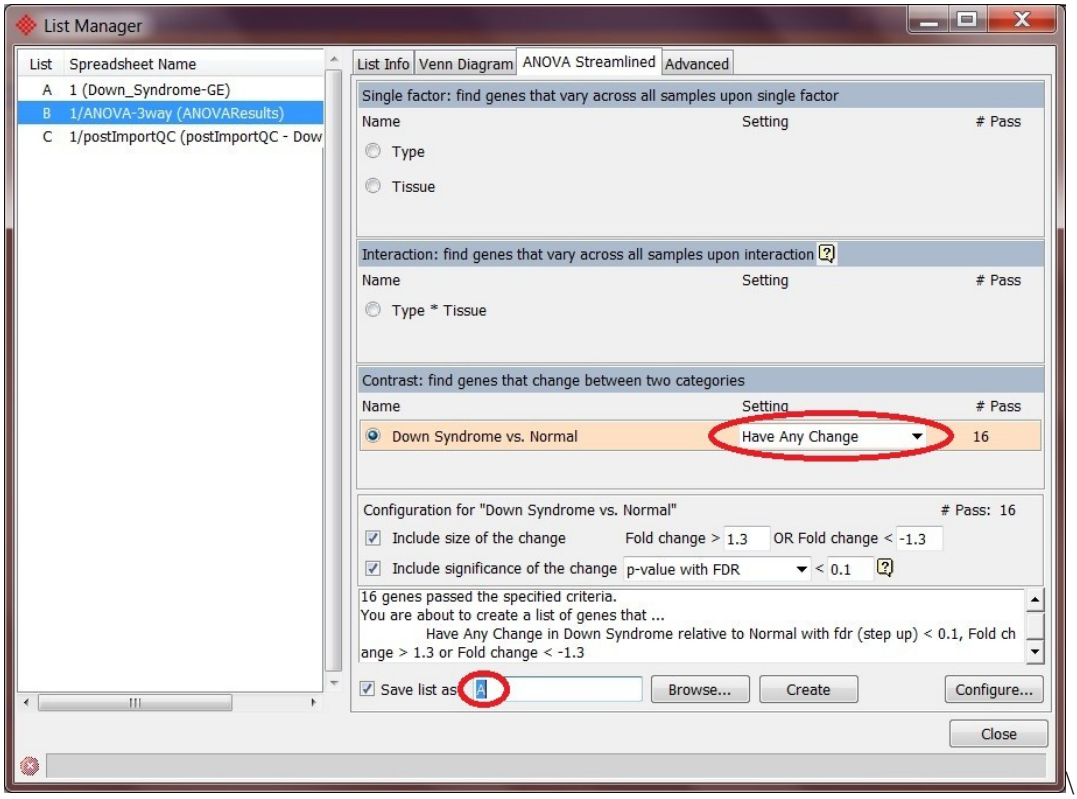


Figure 24: The List Manager dialog for creating a gene list with fold changes above 1.3 or below -1.3 with a False Discovery Rate (FDR) < 0.10

The spreadsheet *Down_Syndrome_vs_Normal (A)* will be created as a child spreadsheet under the *Down_Syndrome-GE* spreadsheet.

This gene list spreadsheet can now be used for further analysis such as hierarchical clustering, gene ontology, integration of copy number data, or exportation into other data analysis tools such as pathway analysis.

You should take some time creating new gene list criteria of your own to become familiar with the *List Manager* tool in Partek® Genomics Suite™. For more information, you can always click on the button.

Hierarchical Clustering

The gene list in spreadsheet *Down_Syndrome_vs_Normal (A)* can now be used for hierarchical clustering to visualize patterns in the data.

- Under the *Visualization* section in the *Gene Expression* workflow, select **Cluster based on significant genes**
- The next dialog asks you to specify the type of clustering you want to perform. Select **Hierarchical Clustering** and select **OK**
- Choose the **Down_Syndrome_vs_Normal (A)** spreadsheet under the *Spreadsheet with differentially expressed genes* (Figure 25)

- Choose the **Standardize – shift genes to mean of zero and scale to standard deviation of one** under the *Expression normalization* panel. This option will adjust all the gene intensities such that the mean is zero and the standard deviation is 1
- Select **OK**

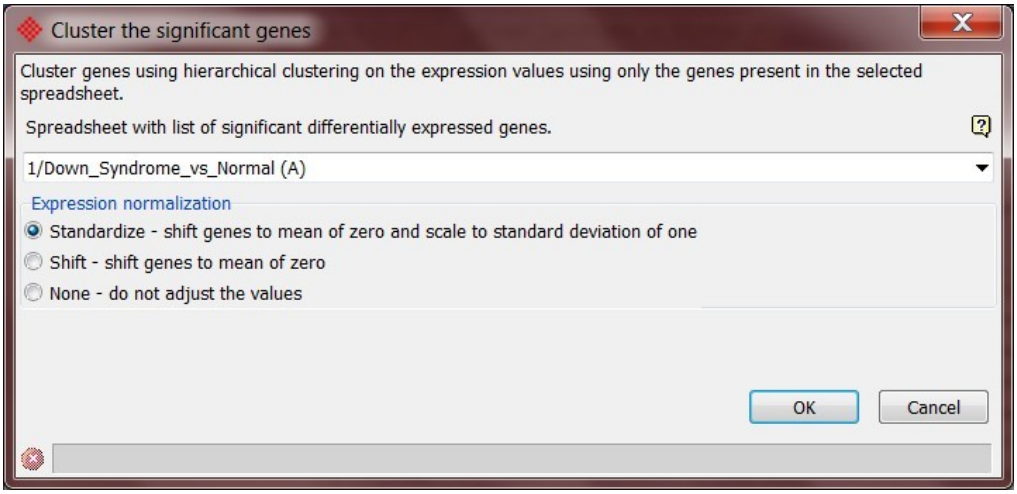

Cluster the significant genes
Cluster genes using hierarchical clustering on the expression values using only the genes present in the selected spreadsheet.
Spreadsheet with list of significant differentially expressed genes.
1/Down_Syndrome_vs_Normal (A)
Expression normalization
Standardize - shift genes to mean of zero and scale to standard deviation of one
Shift - shift genes to mean of zero
None - do not adjust the values
OK
Cancel

Figure 25: Cluster the significant genes dialog

The resulting graph (Figure 26) illustrates the standardized gene expression level of each gene in each sample. Genes, which are unchanged, are displayed as a value of zero and colored gray. Up-regulated genes have positive values and displayed in red. Down-regulated genes have negative values and displayed as blue. Each gene is represented in one column, and each sample is represented in one row. We can see how the Down syndrome samples clustered together as shown in Figure 27.

For more information on the methods used for clustering, you can refer to **Chapter 8: Hierarchical & Partitioning Clustering** *in Help > User's Manual*. For a tutorial on configuring the clustering plot, please refer to the user guide that can be downloaded from: here or from *Help >On-line Tutorials > User Guides.*

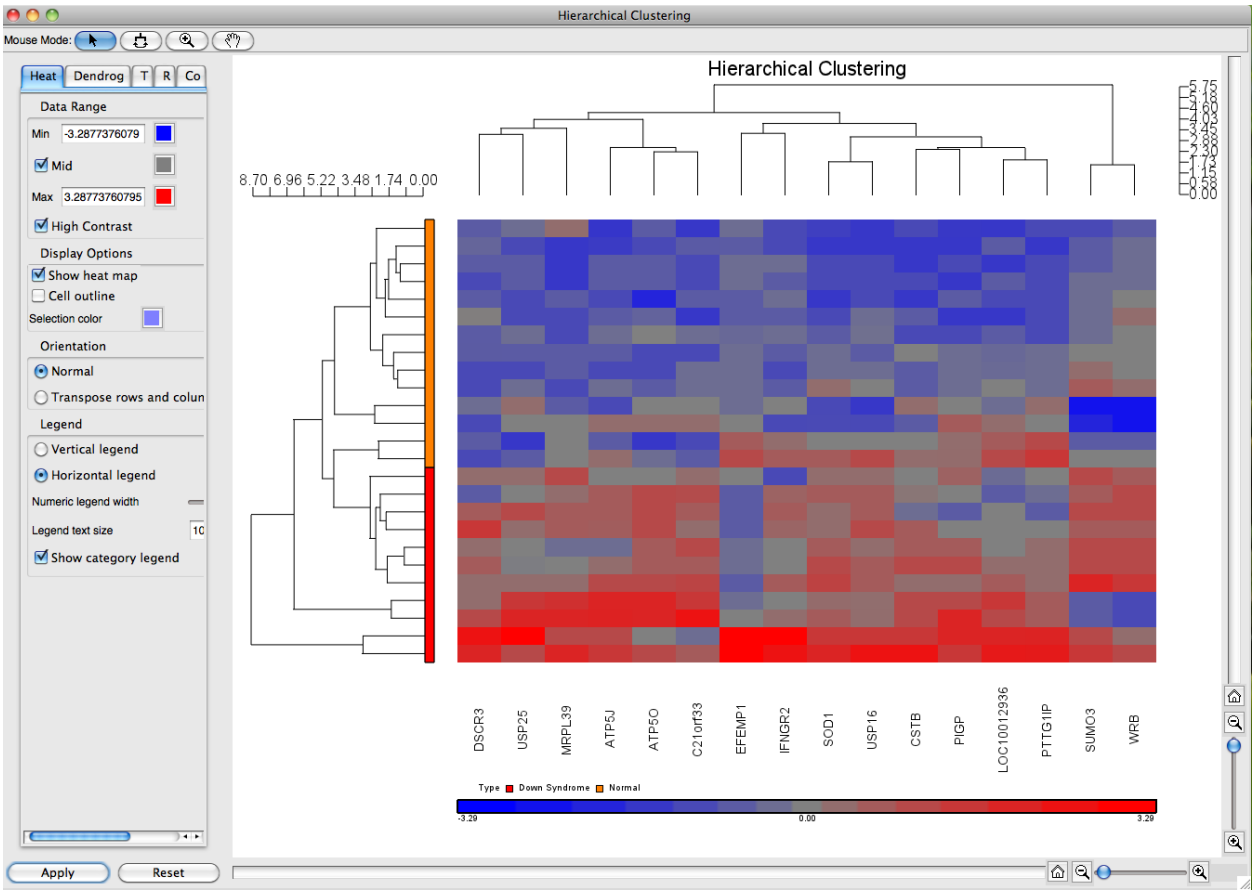


Figure 26: Hierarchical Clustering results

Adding Gene Annotation

In the previous steps, after the data was imported, the GeneChip® annotation file was linked to the data, so now the results spreadsheets will automatically be linked to the annotation file. More information can be added to the genes in new columns if the genes are laid out on rows like in the ANOVA or gene list spreadsheets. For example, if you want to add additional annotation to the gene list A, then:

- Right click on the second column header **(*2. ProbesetID*)** in the *Down_Syndrome_vs_Normal (A)* spreadsheet and select **Insert Annotation** from the pop-up menu (Figure 27)
- Select the **Chromosomal Location** under the *Column Configuration* panel. Leave everything else as default and click **OK** (Figure 28)

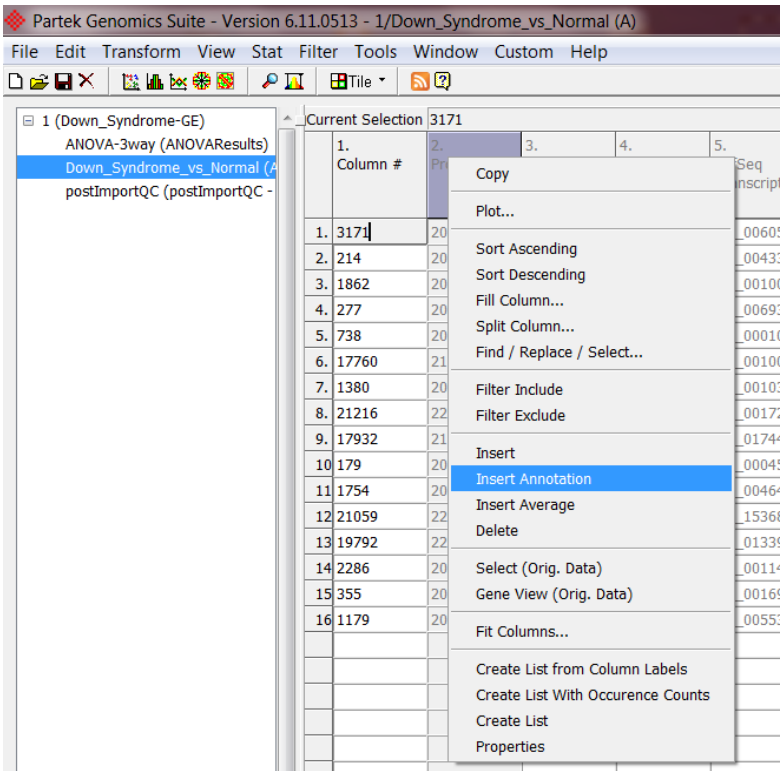


Figure 27: Inserting an annotation column in the gene list result spreadsheet

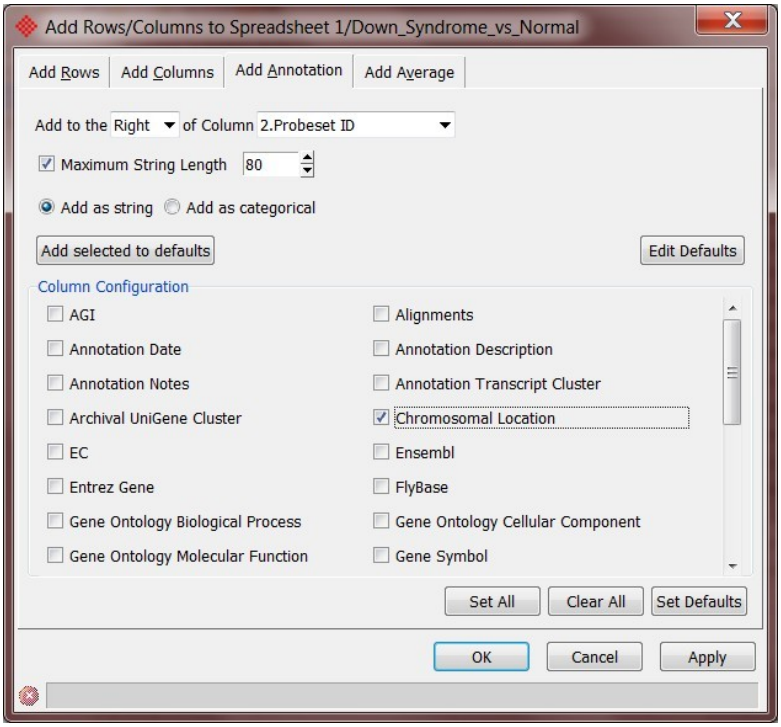


Figure 28: Adding Chromosomal Location to each gene in the gene list spreadsheet

Of the 16 genes of the *Down_Syndrome_vs_Normal (A)* spreadsheet, 14 genes are on chromosome 21.

- Right click on the first row header and select **Probe Set Details** to get more information about that gene (Figure 29)

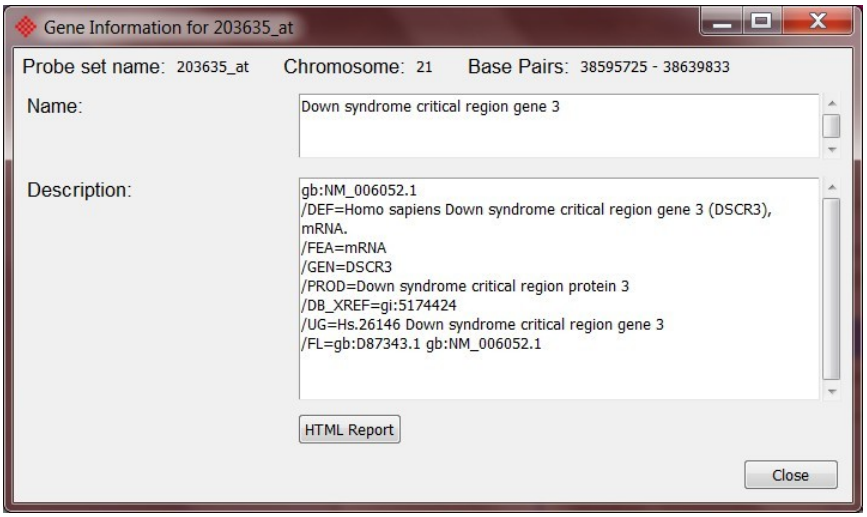


Figure 29: Getting gene information for Probeset_ID 203635_at

- To get a dot plot of a specific gene, right click on the row header and select **Dot Plot (Orig. Data)** from the pop-up menu (Figure 30)

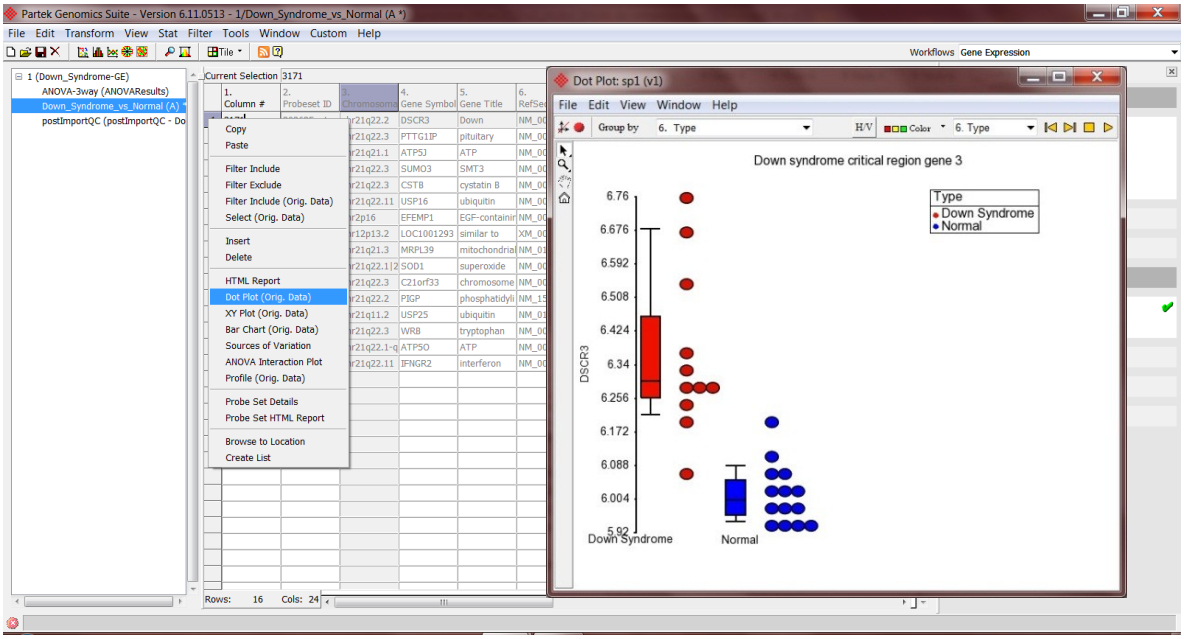


Figure 30: Dot plot of the most differently expressed gene between Down syndrome and normal. Each dot is a sample. The Y-axis represents the expression value of the gene; the X-axis represents different Types. The dots and the box & whiskers are colored by Type

In the plot, each dot is a sample of the original data. The Y-axis represents the $\log_2$ normalized intensity of the gene and the X-axis represents the different types of samples.

The median expression of each group is different from each other. In this example, the median of the Down syndrome samples ≅ 6.3, but the median of the normal samples is approximately 6.0. The line inside the Box & Whiskers represents the median of the samples in a group. Mousing over the Box & Whiskers plot will show this information.

Generating Gene Lists from a Volcano Plot

Next we will generate a list of genes that passed a p-value threshold of 0.05 and fold-changes greater than 1.3 using a volcano plot.

- Ensure that the *1/ANOVA-3way (ANOVAResults)* spreadsheet is selected as this is the spreadsheet we will be using to create the gene list
- Select **View > Volcano Plot** from the Partek® Genomics Suite™ main menu
- Set *X Axis (Fold-Change)* to **13. Fold-Change(Down Syndrome vs. Normal)**, and the *Y axis* (*p-value*) to be **11. p-value(Down Syndrome vs. Normal)**
- *Color by* the gene with the **11. p-value(Down Syndrome vs. Normal)** (*Figure 31*)
- Select **OK**

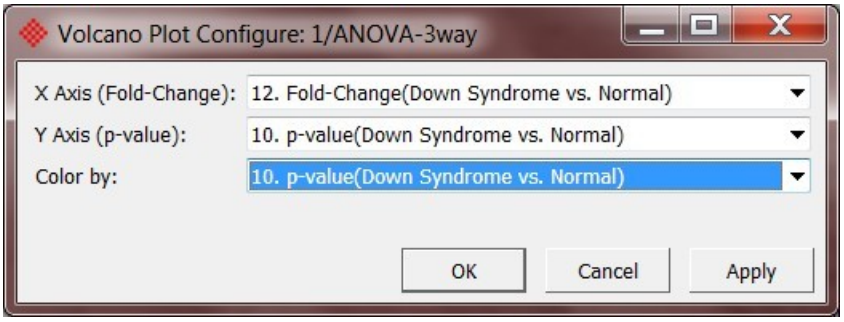


Figure 31: Configuring the volcano plot dialog. X-axis representing the fold change of Down syndrome vs. Normal, Y-axis representing the p-value of this contrast

In the plot, each dot represents a gene. The X-axis represents the fold change of the contrast, and the Y-axis represents the range of p-values. The genes up-regulated in Down syndrome on the right side; genes down-regulated in Down syndrome are on the left of the N/C line. The genes become more statistically significant with increasing Y-axis. The genes that have larger and more significant changes between the Down syndrome and normal groups are on the upper right and upper left corner (Figure 32).

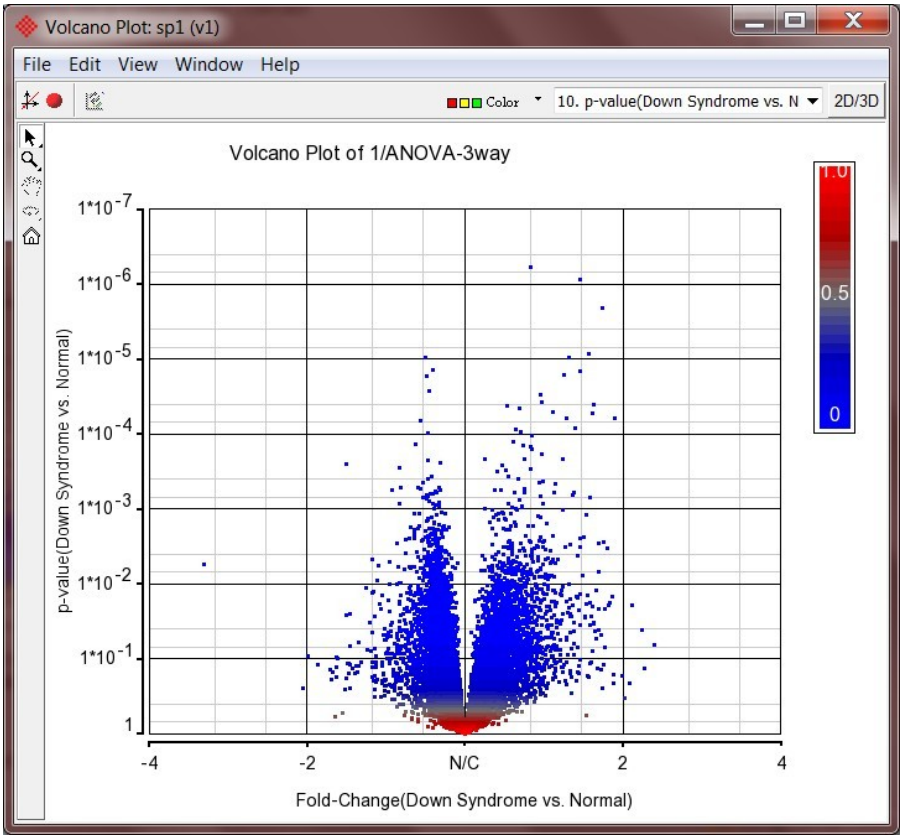


Figure 32: Viewing the volcano plot of Down syndrome vs. Normal contrast. Each dot represents one gene. The X-axis represents fold change, and the Y-axis represents the p-value

In order to select the genes by fold-change and p-value, we will draw a horizontal line to represent the p-value 0.05 and two vertical lines indicating the –1.3 and 1.3 fold changes (cutoff lines)

- Select **Edit > Plot Properties** or the icon ( ) within the *Volcano Plot* viewer
- Choose the *Axes* tab
- Select the **Set Cutoff Lines** button and configure the dialog as in Figure 33Figure 33

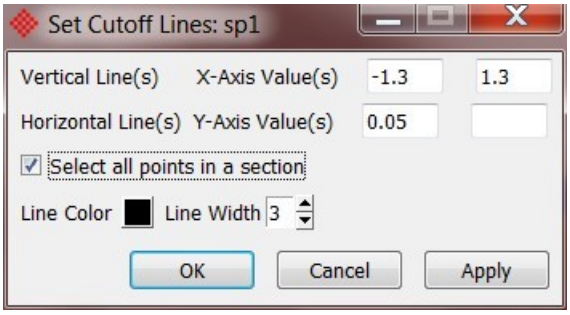


Figure 33: Setting the Cutoff Lines for the volcano plot

- Check **Select all points in a section** to allow Partek® Genomics Suite™ to automatically select all the points in any given section
- Select **OK**

The plot will be divided into six sections. By clicking on the upper-right section, all 271 genes in that section will be selected (Figure 34).

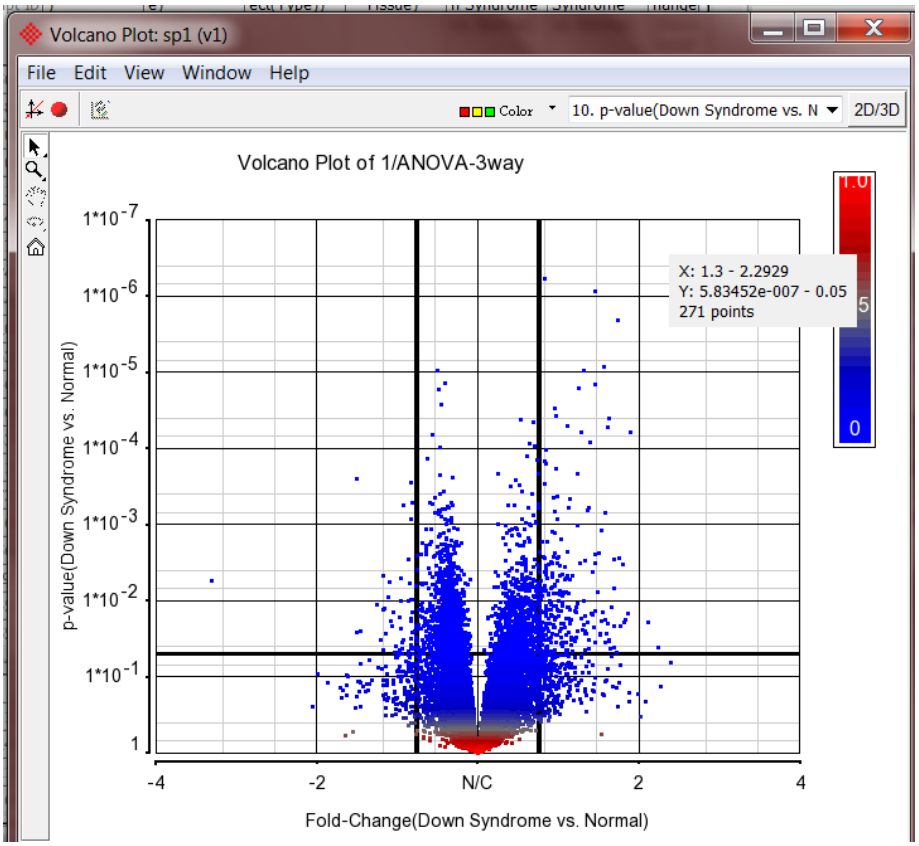


Figure 34: Selecting genes that significantly up-regulated in Down syndrome compared to Normal

- Left-click in any region in the plot to select the region of interest. Right-click on the selected region in the plot and choose **Create List** to create a list including the genes from the section selected. Note that these p-values are uncorrected.

Note: If no column is selected in the parent (ANOVA) spreadsheet, all of the columns will be included in the gene list; if some columns are selected, only the selected columns will be included in the list.

- Specify a name for the gene list and write a brief description about the list (Figure 35). The description is shown when you right-click on the spreadsheet > *Info* > *Comments*

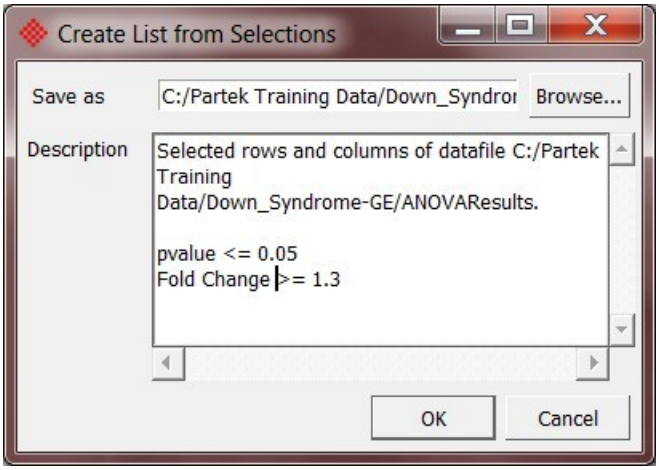


Figure 35: Specifying a name for the gene list

The list can be saved as a text file (*File > Save As Text File*) for use in reports or by downstream analysis software.

End of Tutorial

This is the end of tutorial. If you need additional assistance with this data set, contact the Partek Technical Support staff at +1-314-878-2329 or email us at support@partek.com.

Date last updated: November 5, 2013

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